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in Development

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THE PROBLEM OF CELL INDIVIDUALITY IN DEVELOPMENT

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BEFORE considering its embryological implications, let us first scrutinize the concept of cell individuality in the developed organism, from which it was originally derived. When Schwann says, "Each cell is within certain limits an individual," his qualification "within certain limits" seems to disavow the doctrinary rigidity which some of his followers have injected into the cell theory when they proclaimed that anything that ever happens in an organism is the resultant of individual cell activities. The severe and scornful criticism which these extreme "elementarians" had to take from the opposing camp of extreme "wholists" who wanted the attribute of individuality strictly reserved to the organism as a whole, is too well known to need further comment here. Much of this fight was carried out on philosophical grounds rather than on the factual grounds of observation and evidence. Many arguments advanced for or against the universality of the cell concept were merely rationalizations of beliefs of their authors that an organism ought or ought not to consist of discrete elements.

It is doubtful, for instance, whether the question of contiguity *versus* continuity among the elements of the nervous system would ever have become such a perpetual issue but for the fact that one group needed membranes and the other group "through" connections to explain nervous function as they saw it. Similar preconceptions were allowed to intrude into the problem of plasmodesms, that is, protoplasmic connections between cells. Those who think of protoplasmic communication and control as transportative in nature would rather have them, while transmissive theories can very well do without them. The observed facts themselves conspired with speculations on both sides by affording the expedient of artifacts caused

by histological treatment: If a preparation shows cell bridges, one can not always be sure that they might not be coagulated intercellular matter; and if a preparation shows no cell bridges, one can never be sure that they might not have been present in the living but ruptured by violent contraction during fixation.

As these few examples indicate, there has been a definite tendency to rate cellular individuality by the criterion of morphological discreteness. Insularity of cell behavior seemed to presuppose completely closed boundaries as of an island, and the evidence for this was sought in uninterrupted microscopic delineation. Obviously such an attitude is unwarranted in view of present-day biological knowledge which has stripped the microscopically visible of its former prerogative as vital standard and has pointed us more and more toward the ultramicroscopical and molecular realm. A submicroscopical boundary along which colloidal and other physico-chemical properties of the protoplasm change is no less real and physiologically effective, even though the microscope fails to reveal its existence. Nevertheless, some of the old practices are still with us, and to this day much of the discussion of cell individuality continues to revolve around microscopical arguments.

From this angle, however, the question is unsolvable. There is as much evidence for the existence of discrete, well-demarcated cell individuals as there is for that of plasmodia, large protoplasmic continua containing numerous nuclei but no visible cell limits to fence off nuclear domains. Free migratory cells are clearly unicellular individuals; but the heart muscle is clearly a syncytium; so is the blastoderm of the insect egg—to mention only a few prototypes. The occurrence of both protoplasmic continuity and protoplasmic fragmentation has been amply demonstrated. But more than that: it has been demonstrated that either condition can change into the other. Morphological delineation has thus turned out to be a rather inconstant character and by no means a true

test of cellular individuality. Cell bridges can break and reform; symplasms can divide up into cells; and cells can merge again into syncytia. Let us choose three examples to illustrate these facts, one from the lowest and the other two from the highest group of animals.

(1) In continuation of earlier studies by Wilson, Galts-off and others, Brondsted has recently made a thorough reinvestigation of the problem of cellular individuality in the reconstitution and germination of sponges. His observations leave no doubt that cell discreteness is of a transitory character, which comes and goes according to circumstances. Repeatedly, cells merge into large plasmodial masses, thereby losing their outlines, and later emerge again as individualized, well-circumscribed units. Free circulation of granules and other inclusions between the formerly isolated cell body and the ground substance into which it has opened, attests to the disappearance of morphological cell boundaries in the act of fusion.

(2) The syncytial character of the mesenchyme of vertebrates has been asserted by Rohde, Hueck, Studnička and others; again it has been recorded that under certain conditions nucleated parcels of protoplasm emancipate themselves from the syncytial continuum as mobile cells which can probably later become reincorporated in the common plasmodium (v. Möllendorff).

(3) Perhaps in no other tissue has the dogma of the morphological discreteness of the cellular individual been so vigorously defended as in the nervous system. As for the embryonic origin of the nerve fibers, the neurone doctrine has come out victorious, inasmuch as the neurite of each nerve fiber has been found definitely to be the product of a single discrete nerve cell. However, evidence of secondary protoplasmic anastomoses of the individual units keeps steadily accumulating. To the best of our histological knowledge, particularly according to Boeke, the terminal branches of a motor nerve fiber pass into the protoplasm of the muscle fiber without morphological interruption. Similarly, the connection between a cutaneous

nerve fiber and its sensory end organ has been described and depicted as intraprotoplasmatic. Anastomoses among nerve fibers were observed in the living object by Speidel and in tissue culture by Guiseppe Levi, who otherwise is one of the staunchest supporters of the individuality of the neurone. In nerve regeneration the newly outgrowing nerve fibers merge with the protoplasmic syncytium of the so-called cords of Büngner and only later become set off from the matrix and from one another by insulating sheaths.

Apparently, cell contour is a much more variable character than one would have anticipated. It can fade and reappear. But does this upset the concept of cell individuality? I do not think it does. In the studies on sponges quoted above, Brondsted has reported that even after merging into plasmodia, the different types of constituent cells can still be distinguished by differences of their nuclei and perikarya, and when a cell becomes released again, it behaves true to its original kind. Evidently, cellular individuality can survive protoplasmic confluence. We may adjust our picture of the cell to this situation by de-emphasizing the criterion of delineation. The only definitely discrete element in a cell is the nucleus, and since each nucleus keeps protoplasm within a certain radius under its control, protoplasmic territories have the value of cells, no matter whether their boundaries are marked by visible surfaces or merely by a change of physiological properties along the border. Any change in the colloidal consistency of the protoplasm attended by biochemical and bioelectrical differences will necessarily produce a definite orientation of ultramicros along the boundary and create some sort of physiological barrier and some degree of physiological isolation.

Within these limits, the cell is an individual, anatomically and physiologically speaking. But what about the cell in development? Let me briefly outline the crux of this problem also.

At the end of development we are confronted with a

unitary organized system, called an "organism," which, at the same time, is a collective of cells. At the beginning of development we find just one primordial cell—the egg. We call a system "organized" when its multiple elements appear in typical diversity, typical spatial distribution and typical temporal order. The elements are subordinated to this order and their freedom is restricted by it; hence, the order is a supra-elemental property of the system. In the developed system, "organism," the cells represent the elements; hence, organization is a supra-cellular property. But the primordium of the organism—the egg—does not consist of cells. Now, there arises a dilemma. Either the egg already possesses supra-cellular organization of the same order as the later body—then it is not just another cell, but an uncellulated organism; or it is merely a cell like others—then it can not be at the same level of organization as the later body. In this case, development would create organization of a higher order. It is to this latter view that the cell theory has committed itself. In the words of Schwann, "the individual cells so operate together in a manner unknown to us as to produce a harmonious whole," and the stress lies on "produce." Cells springing forth from repeated divisions would join hands, as it were, as equal participants in the building up of an organization all of their own making. The organism would be synthesized by progressive integration of cells into higher units, tissues, organs and the body as a whole. Cells would form the organism.

This view has met with vigorous opposition, culminating in a number of pronouncements about the inadequacy of the cell theory of development. In his address at the World's Columbian Exposition, Whitman argued the case in the most trenchant manner. One must realize the philosophical implications of the problem. If organization was to be accepted as something created *de novo* in every ontogeny, some principle had to be invoked which could mold order out of chaos, and the resort to vitalistic agents, such as Semon's "Mneme" and Driesch's "Entelechy,"

was a logical outcome. Faced with the alternative, the assumption of some primordial organization inherent to the egg seemed to many a much more palatable solution. Thus, the egg was vested with organizing powers representing the supra-cellular organization of the later organism, and research was directed toward the establishment of external signs of this organization. The egg and the young germ were considered as primarily integrated wholes within which parts gradually arise by individuation. At no time would the cells constitute independent units, but from the very beginning they would be subordinated to the actions of the organism as a whole. The cells would not form the organism, but the organism would break up into cells.

Clearly the two opposing views represented a modern edition applied to organization of the old antithesis: epigenesis *versus* preformation. Epigenesis of organization was the claim of the "egg-equals-cell" theory, while preformation of organization was the tenet of the "egg-equals-organism" doctrine. The latter soon gathered momentum from experimental evidence. Lillie showed that activated eggs of a worm, *Chaetopterus*, when prevented from cleaving into cells as in ordinary segmentation, still underwent a considerable degree of differentiation, involving development of parts within the protoplasmic continuum of an undivided egg. Localized differentiations of the egg cytoplasm of ctenophores, annelids, molluscs, insects and amphibians became known which imparted definite substantial and dynamic properties upon the cells to which they happened to become apportioned during cleavage. The cellulation of the egg was gradually recognized as a sort of epiphenomenon superimposed upon the differentiation of the germ rather than instrumental in its production. More and more one became impressed by the fact that the organization of the germ as a whole has stability as such, regardless of the extensive fluctuations to which its cells are subjected in nature and experiment. The individual cells began to appear as slaves, rather than

bosses, of the organism. The existence of individual cells as units was still acknowledged, but their rôle in embryonic organization was strongly de-emphasized.

As so often happens, however, in the wake of this sound reaction to exaggerated claims of the cell doctrine, an equally intransigent anti-cell doctrine raised its head. It tried to deny cellular individuality altogether and advanced a veritably totalitarian concept of development. Cells were ignored. The mass of the developing organism was considered as clay in the hands of the sculptor, passively submitted to molding forces which neither respect internal boundaries, nor admit of constitutional autonomy of individual units. If to the extremists of one side the individual cell was all and everything, to the advocates of the other extreme the organism as a whole appeared from the beginning in unchallenged control, cells or no cells.

Experimental embryology has, on the whole, steered clear of the two extremes. But it was difficult for the issue to find its proper level so long as one put the problem in terms of an alternative: Is the egg a cell or an organism? Is development epigenetic or preformed? Do the cells establish the properties of the developing organism or does the organism determine the properties of its cells? And so on. As we now see them, these questions are about as pertinent as if one asked: Has the face of the earth developed by volcanism or by erosion? The face of the earth is a highly complex affair, and so is its development. So also is the development of an organism. The time has passed when one could speak of development as if it were a single simple unitary phenomenon, like lightning or crystallization or the casting of a mold. Experimental analysis has revealed that what, in one word, we plainly call "development," is in reality an intricate combination of innumerable component processes, diverse and often disparate in character, which merely simulate oneness in that they all affect the identical material system—germ. Of course, it has been customary all along to single out growth (meaning increase in mass) or differentiation (in-

crease in diversity) or morphogenesis (elaboration of shape) and the like; but there has been a general feeling that all these features are manifestations of a common principle, and that to separate them was pardonable only as an act of mental abstraction. The truth, however, is that they are essentially separate phenomena, and, in fact, each one in itself highly composite. Nuclear division, cell growth, cell division, cell aggregation, movements of cell complexes, differential growth, cytological differentiation, polarity, orientation—these are only a modest selection from the list of component phenomena into which we have learned to decompose development.

The revelation of the multiplicity of developmental processes and mechanisms has been a sad disappointment; for it has removed all hope of a general, comprehensive and universal formula of development. At the same time, it compels us to ask every question which formerly was aimed at development in general, separately for each one of its manifold components. We no longer ask: "Is development epigenetic or preformed?", but focus on a single contributory phase, asking: "How much of it is due to epigenetic and preformed conditions?", only to find that the answer varies with the object. It is this abandonment of the unitarian claim which has rendered us immune to both the strictly elementarian and the strictly totalitarian view, and which has steadied our picture of the relative rôle of cell and organism in development. Instead of a sweeping generalization, we expect a precise description of just how much of a given developmental phenomenon is due to active participation of the cells and how much to effects of supra-cellular order; what does a cell do, and what is being done to it, in a given phase of development? These are questions with which one can deal in matter-of-fact fashion, without even touching the sore spots of principle.

A few specific cases may serve as examples. Let us consider, first, histological differentiation. Do cells produce specific histological characters by intrinsic capacity

or through external influences? For instance, is the elaborate conducting and contractile apparatus of a cross-striated muscle fiber developed by virtue of a constitutional property of the myoblast cell or can muscular development perhaps be imposed upon any protoplasmic mass by proper influences from its surroundings, as has been claimed by Carey? Observation and experiment have answered in no uncertain terms: Cellular differentiation is founded on innate properties of the cells themselves.

To prove the point, we remove cells from the community of the organism, thus depriving them of possible outside directives. We choose cell groups of an early germ with no manifest signs of differentiation, explant them into an extraneous medium and watch their fate. Morphogenetic development remains poor. But histologically, the explanted cells differentiate with amazing perfection. As Holtfreter and others have shown, they give rise to typical nerve cells, pigment cells, muscle cells, cartilage cells, notochordal cells, goblet cells, pronephric cells, etc. These productions are absolutely definite and discrete, each cell differentiates distinctly into one type or another, and there are no intergradations, hybrids or blends between the established cell types. Obviously, even very young cells "know" how to make a muscle fiber, a neurone, a chromatophore, etc., and we may conclude that the mechanisms for histological differentiation belong to the preformed endowment of a cell.

The same experiments have revealed, however, that cells are by no means single-tracked from the beginning. We know approximately what is to become of any given cell group of an early germ during normal development. Now, one has often noted that cells, when reared in isolation, can deviate considerably from their normal fate. Presumptive nerve cells, for instance, can become muscle cells or chorda cells, and the like. This means, evidently, that each cell of the early germ possesses a definite repertoire consisting of several discrete differentiation po-

tencies. A limited number of clearly circumscribed courses are open to each cell.

In isolation, chance may decide which course is actually followed. But inside the organism, the choice is definite: there, each cell develops in conformity with the character of its surroundings. It becomes a cone or rod when in the retina, a cartilage cell when in the center of a limb bud, and a neurone when in the brain. Intrinsically capable of a variety of performances, the cell receives some definite cue from the locality indicating which trend it is to follow. These cues are decidedly of supracellular origin. Their effects have been beautifully demonstrated by transplantation experiments for which the schools of Spemann and Harrison have become famous. Transplant a young and undifferentiated cell group into the region of the head, and it will form eye or brain; transplant it to the anterior trunk and it will form limb, or further back, kidney; and, transplant it to the rear, and it will form tail—the same cells forming different structures depending on their locations. We may say: “Organizing factors take hold of the cells and direct them to appropriate formations.”

We must be careful not to lapse again into the erroneous metaphor of the sculptor molding clay; let us stress, therefore, that no organizing factor has yet been observed that would have made cells assume histological structures strange to their inherited repertoire. This statement is based on crucial evidence obtained from transplantations between different species and orders of animals. In provoking specific histological characters, organizing influences are bound, therefore, to operate through the cells as their executives, and the specific character of the execution is determined by the properties of the reacting cells. To this extent, differentiation is active cell work. But this is not the whole story: The factors which turn a given cell into a definite histological trend do not, at the same time, fix all the particulars of its future course. Take a nerve fiber, for example. The factor which turns an indifferent epithelial cell into a nerve cell does not, at the same

time, decide the spot at which the nerve fiber will leave the cell body; and the factor which opens the door for the fiber has no control over the further journey of the outgrowing sprout; and, again, the factors which map this course are different from those which decide where it will terminate. To put it drastically, the nerve fiber is elaborated in assembly plant fashion. In some phases of this sequence the individual nerve fiber plays an active role; in others it behaves purely passively. The original outgrowth of the sprout is free, guided presumably by oriented traffic routes of the surrounding body. But once the free tip has become hitched to a peripheral migratory cell, it is taken in tow and dragged to a destination no longer of its own choosing. It is not at all easy to tell how much of the winding course of a nerve fiber is due to active orientation and how much to passive distortion.

The same holds for cell shape in general. Part of it can be ascribed to autonomous transformations of the individual cell body, the rest to passive deformation caused by pressure, growth and spatial limitation on the part of the cell collective. With cell movements it is the same story: Free cells may aggregate in response to a local stimulus and thus form a crowd whose further growth and movement, as a whole, sweep the participant elements along without leaving them much further individual choice. Similarly, erstwhile free cells which secrete a cementing substance thereby imprison themselves and become subjected to all the dislocations of their common matrix. An opening or canal may be formed either by the active recession of the cells lining the prospective lumen or by the passive destruction of cells with subsequent resorption. When one sees pigment cells arranged in regular geometric tracts, one suspects that they have been forced into this alignment by the topography of their surroundings. But how much the pattern is really of their own making has been shown by Twitty when he interchanged the source of the pigment-forming cells between two species of distinctly different color patterns: the transplanted cells

assumed the distribution characteristic of the species of their origin rather than that of the host body. I would venture to say that what the individual cells actually bring into the deal in this instance is a tendency either toward dispersion or toward aggregation; whereas the loci of aggregation in the latter case are presumably a matter not of the cells themselves, but of their matrix, so that the resulting pattern would, again, be of composite origin.

I have deliberately dwelt on these varied examples, in order to make clear that practically every step in development reveals the cell in a double light: partly as an active worker and partly as a passive subordinate to powers which lie entirely outside of its own competence and control, *i.e.*, supra-cellular powers. Now, it is perfectly true that some of these latter result from interactions of cell individuals and are, therefore, of cellular origin. But it is equally true—and the findings of experimental embryology are one rich store of evidence for our assertion—that many of them are supra-cellular from the beginning. They are those organizing conditions through which the fate of the individual cells—undecided, as we have seen, at first—is guided, controlled and progressively fixed. They are definite at a time when the individual cell fate is still indefinite. They impose order upon what otherwise would be an anarchic cell chaos. They are inherent properties of the living system, germ, as a whole, in contradistinction to the inherent properties of its constituent cells of which we have spoken before.

One frequently refers to these organizing entities under the term of "fields." Their existence can be traced back to the egg. In fact, just as there is a continuity of cells from the egg to the organism through successive cell divisions, so there is continuity between the primordial organizing fields present in the undivided egg and the localized fields of the later germ. Primordial fields segregate progressively into more restricted fields, and, furthermore, induce new fields in neighboring areas of the germ. Thus,

the organizing principles of a germ have an ontogenetic history of their own which is not cell history. Their possession marks the egg as an entity of the rank of the organism; this, in answer to a question put above. Their development is a matter of the developing system as a continuum, like tensions, currents, potentials, and the like, and they pay no heed to cell boundaries, although sooner or later the intricate interplay between them and the cells sets in, of which we have spoken before.

The existence of these primordial organizing principles in the egg has been firmly established by modern experimental embryology. No pure cell theory derived from the developed organism can embrace them, unless by a vicious circle.

In conclusion, we may say that the cell theory is correct: The egg is a cell and it gives rise to all the successive cell generations which contribute to the organism. But the organismic theory is likewise correct: The egg is also an organism, and it passes its organization on continuously to the germ and the body into which it gradually transforms. Only this dual concept seems to fit the facts, as we see them at present. To be consistent, we should supplement Virchow's well-known tenet of the cell theory: "Omnis cellula e cellula," by its counterpart: "Omnis organisatio ex organisatione." If the former denies spontaneous generation of living matter, the latter denies spontaneous generation of organization. In admitting this, we merely paraphrase what Whitman has called the "continuity of organization." But within these specified limits the cell, even in development, is still, as Schwann has said, an individual.